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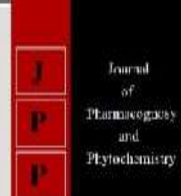

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Pharmacognostical and phytochemical investigations of a terrestrial and an aquatic plant

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Abstract
This paper describes the Pharmacognostical and Phytochemical screening of selected plants that is *Procris repens* and *Sargassum duplicatum*. *Procris repens* (Lour.) B.J.Conn & Hadiah belonging to the family *Urticaceae* commonly called as 'Trailing Watermelon Begonia' and 'Wavy Watermelon Begonia'. *Sargassum duplicatum* J. Ag. is one of the main seaweeds in the marine ecosystem that has been used as a source of medicine among brown seaweeds belonging to the family *Sargassaceae*. This review paper discusses in detail study of Pharmacognostical and Phytochemical screening. In Pharmacognostical study discusses the microscopy and macroscopy of selected plants. Here the macroscopical study describe the characters like colour, odour, taste, size, shape, extra features of the leaves. The microscopical study describe the fresh leaves were subjected to various anatomical studies. The sections of leaf specimen were taken using sharp blade and stained with various staining reagents like phloroglucinol-HCL, iodine, safranin and observed under microscope. Phytochemical screening of *Procris repens* found to contain alkaloids, steroids, triterpenoids, saponins, glycosides, tannins, phenolic compounds and flavonoids while in addition to it *Sargassum duplicatum* extract also contain carbohydrate also.

Keywords: *Procris repens*, sargassum duplicatum, microscopy, macroscopy, phytochemical

Introduction
Procris repens belonging to family *Urticaceae* is commonly known as Trailing watermelon begonia. Almost all the parts ie, stem, leaf, flower are used for different type of ailments. It is a small herbaceous creeper, native to Southeast Asia. It typically grows to about 15 cm in height and can spread up to 60 cm wide through rooting stems. The leaves are elliptic to oblong, with wavy edges, measuring up to 6.4 cm long, displaying a variable gray-green colour with darker edges and a lighter patches. *Procris repens* produces white or pink tiny flowers with a tinge of silver, and arranged in branched clusters and is unisexual, monoecious. It has polycarpic flowering habit. It possesses fibrous root system that aids in nutrient and water absorption. It is used by Malays for poulticing boils, swollen areas, and the abdomen when it is painful. A decoction from the plant is used for rheumatism. *Procris repens* (Lour.) B. J. Conn and Hadiah (*Urticaceae*) is traditionally used in folk medicine for the treatment of skin injuries and respiratory conditions. Whole plant of *Procris repens* was used to treat icterus, acute and chronic hepatitis, and allergic dermatitis in Chinese medicine.

Fig 1: *Procris repns*

Common Names
- Trailing Watermelon Begonia.
- Wavy Watermelon Begonia.
- Satin Creeper.
- Rainbow Vine.
- Sisek Naga.

Taxonomical classification

Kingdom	Plantae
Phylum	Tracheophyta
Class	Equisetopsida
Subclass	Magnoliidae
Order	Rosales
Family	Urticaceae
Genus	*Procris*
Species	*Procris repens*

Botanical descriptions of *Procris repens* (Lour.) B. J. Conn and Hadiah

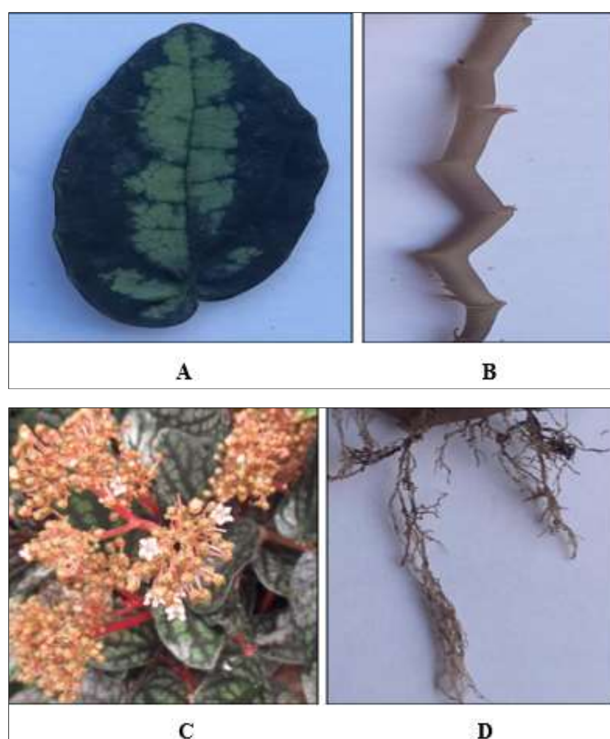

Fig 2: (a) Leaf (b) Stem (c) Flower (d) Root

Procris repens belonging to family *Urticaceae* is commonly known as Trailing watermelon begonia. Almost all the parts ie, stem, leaf, flower are used for different type of ailments. It is a small herbaceous creeper, with prostrate ground-hugging habit, up to 15 cm tall, spreading or trailing up to 60 cm. *Procris repens* exhibits a low-growing and trailing growth habit. Its stems sprawl along the ground, creating a prostrate and spreading form. This growth pattern allows the plant to form dense mats of vegetation, contributing to its ecological role in stabilizing soil and providing ground cover. The stems are slender, wiry, and prostrate, rooting at the nodes. It has alternate, fleshy leaves have toothed or wavy leaf blades that are elliptic to oblong, and sometimes broadly egg-shaped, asymmetrically cordate based, and 2.5-10 by 2-5 cm. Procris repens produces white or pink tiny flowers with a tinge of silver, and arranged in branched clusters and is unisexual, monoecious. It has polycarpic flowering habit. It possesses fibrous root system that aids in nutrient and water absorption. It is used by Malays for poulticing boils, swollen areas, and the abdomen when it is painful. A decoction from the plant is used for rheumatism. *Procris repens* (Lour.) B. J. Conn and Hadiah(Urticaceae)is traditionally used in folk medicine for the treatment of skin injuries and respiratory conditions. Whole plant of *Procris repens* was used to treat icterus, acute and chronic hepatitis, and allergic dermatitis in Chinese medicine.

Chemical constituents: Alkaloids, glycosides, phenols, flavonoids, saponin, tannins, steroids and triterpenoids.

Uses: It is used to treat icterus, acute and chronic hepatitis and allergic dermatitis in Chinese medicine. It is used in folk medicine for the treatment of skin injuries, nerve-related disorders, gastrointestinal complaints and respiratory conditions.

Sargassum duplicatum

Sargassum duplicatum belonging to the family *Sargassaceae*. Mainly found in Rameswaram, Tamil Nadu.

Fig 3: *Sargassum duplicatum*

Taxonomical classification

Kingdom	Chromista
Subkingdom	Harosa
Infrakingdom	Heterokonta
Phylum	Ochrophyta
Class	Phaeophyceae
Subclass	Fucophycidae
Order	Fucales
Family	Sargassaceae
Genus	*Sargassum*
Species	*Sargassum duplicatum*

Synonyms
- *Sargassum duplicatum var.duplicatum* J. Ag.
- *Sargassum duplicatum var.moumeensis* Grunow

Botanical Description

Stem terete, up to 11 mm high. Primary branches up to 65 cm length, cylindrical at base, slightly compressed at lower to middle part, up to 3.0 mm in width, up to 2.5 mm in depth, with smooth surface. Primary leaves broader elliptical to lanceolate up to 43 mm in length, up to 18 mm in width. Secondary leaves narrower elliptical to lanceolate, up to 40 mm in length up to 14 mm in width, base cuneate, apex obtuse, margin entire to small dentate, midrib immersed, vanishing below the tip of the leaves, scattered small,

conspicuous cryptostomata. Vesicles compressed ellipsoid or obovoid, up to 10 mm in length, with sharp spines or appendages or crown leaves, stalks terete, up to 3 mm in length, shorter than the vesicles. Plant dioecious. Female receptacles slightly once divided, racemosely arranged. Male receptacles unknown. Mostly brown or dark green in colour.

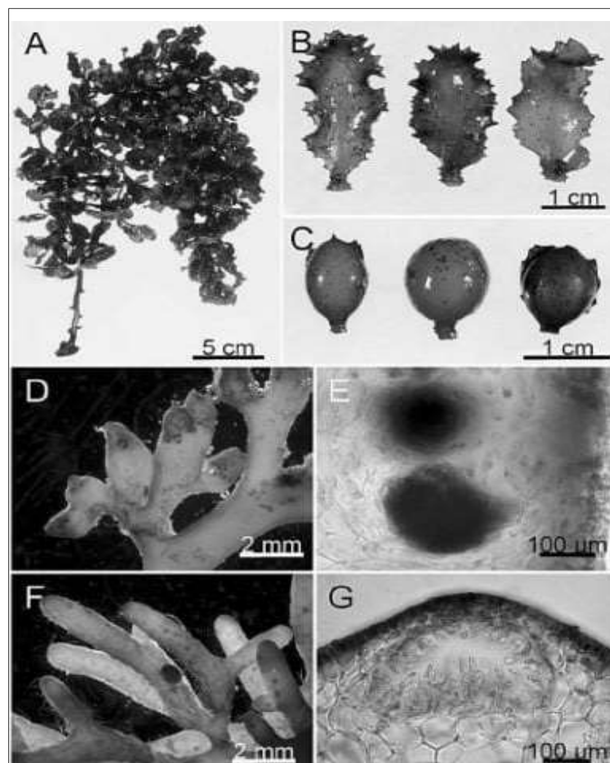

Fig 4: A: Whole habit. B: Leaves. C: Vesicles. D:Female receptacle. E: Transverse section of female receptacle showing a conceptacle. F: Male receptacle. G: Transverse section of male receptacle showing a conceptacle

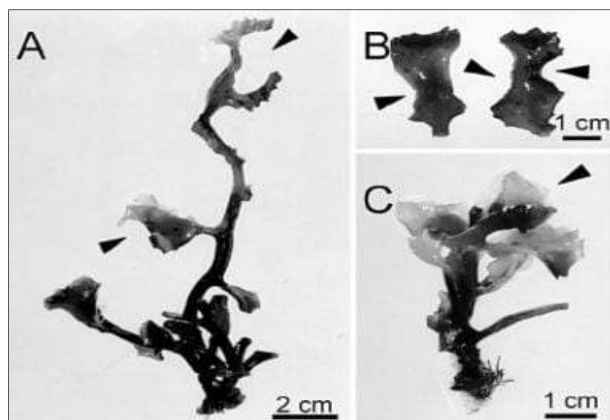

Fig 5: A: Lacking thalli. B: Leaves. C: Bleached leaves

Uses of *Sargassum duplicatum*

- Antidiabetic
- Anticancer
- Anti- macrofouling agent
- cytotoxic
- Wound healing activities

Materials and Methods

Pharmacognostical studies

Macroscopy and microscopy of fresh leaves

Macroscopical characters

The macroscopical characters like colour, odour, taste, size, shape, extra features of the leaves were studied, and the results were tabulated.

Microscopical studies

The fresh leaves were subjected to various anatomical studies. The sections of leaf specimen were taken using sharp blade and stained with various staining reagents like phloroglucinol-HCL, iodine, safranin and observed under microscope.

Preparation of powder

The collected leaves of *Procris repens* and *Sargassum duplicatum* were washed with running tap water to remove adhering materials. Then the leaves were dried under shade. The dried leaves were pulverized mechanically into coarse powder. The coarse powder (passed through sieve no. 18 and retained on sieve no. 60) was collected and used for Pharmacognostical and Phytochemical studies. The fine powder was used for Powder Microscopy.

Powder microscopy

Majority of the crude drugs for commercial purpose are available in powder form. So, the powder microscopic studies give the anatomical characters of fragmented crude drugs. Glycerine mounted temporary preparations were made for powdered leaves.

Determination of physicochemical constants

Ash values

The ash of any organic material composed of their non-volatile inorganic components. Controlled incineration of crude drugs results in an ash residue consisting of an inorganic material (metallic salts and silica). Ashing involves oxidation of the components of the product. This value varies within wide limits and is therefore an important parameter for the purpose of evaluation of crude drugs. A high ash value is indicative of contamination, substitution, adulteration or carelessness in preparing the crude drug for marketing. In certain drugs, the percentage variation of the weight of ash from sample to sample is very small and any marked difference indicates a change very small and any marked difference indicates a change in quality. Unwanted parts of drugs can raise the ash value. More direct contamination like sand or earth is immediately detected by the ash value.

A. Total ash

Total ash is the measure of total amount of material produced after complete incineration of the ground drug at as low temperature as possible (about 450 °C) to remove all carbons. Total ash usually consists of carbonates, phosphates, silicates and silica which includes both,

Physiological ash – derived from the plant tissue itself

Non-physiological ash – residue of the adhering material to the plant surface, e.g., sand and soil.

Procedure

Incinerated about 3 g accurately weighed powdered drug in tared platinum crucible at a temperature not exceeding 450 °C until free from carbon. Cooled and weighed, repeated to get constant value. Percentage total ash was calculated with reference to the air-dried drug.

B. Acid-insoluble ash

It is the ash obtained after extracting the total ash with Hydrochloric acid.

Procedure

The total ash obtained was boiled with 25 ml of 2 M HCl for 5 minutes. The insoluble matter was collected on an ash less filter paper (Whatman - 41), washed with hot water. Transferred the filter paper containing insoluble matter to the original crucible, ignited, weighed and calculated the percentage acid-insoluble ash with reference to the air-dried drug.

C. Water soluble ash

It is the part of total ash content which is soluble in water. It is a good indicator of either water-soluble salts in the drug or incorrect preparation. It is the difference in weight between the total ash and the residue obtained after treatment of total ash with water.

Procedure

The total ash obtained was boiled for 5 minutes with 25 ml of water; collected the insoluble matter on an ash less filter paper, washed with hot water, and ignited for 15 minutes at a temperature not exceeding 450°C. Subtracted the weight of the insoluble matter from the weight of the ash; the difference in weight represents the water soluble ash. Calculated the percentage of water-soluble ash with reference to the air-dried drug.

D. Sulphated ash

Procedure

Heated a platinum crucible to redness for 10 minutes, allowed to cool in a desiccator and weighed. Put 3g accurately weighed powdered drug, into the crucible, ignited gently at first, until the substance is thoroughly charred. Cooled, moistened the residue with 1 ml Sulphuric acid, heated gently until white fumes are no longer evolved and ignited at 800 ± 25°C until all black particles have disappeared. The crucible was cooled, few drops of Sulphuric acid was added and again heated. The ignition was carried out as before, cooled and weighed to get a constant weight. Calculated the percentage of sulphated ash with reference to the air-dried drug.

Extractive values

It is the number of active constituents in a given amount of medicinal plant material when extracted with a particular solvent. The composition of phytoconstituents in that solvent depends upon the nature of the drug and solvent used. The use of a single solvent can be the means of providing preliminary information on the quality of a particular drug sample; for example, in a drug where the extraction procedure for the constituents commence with water as solvent, any subsequent aqueous extraction on the re-dried residue will give a very low yield of soluble matter. Water soluble, alcohol soluble and ether soluble extractive values are an indicative of poor quality, adulteration with any unwanted materials or incorrect processing of the crude drug during the process of drying, storage etc.

A. Alcohol soluble extractive

Macerated 5 g of air-dried, coarsely powdered drug with 100 ml of 90% ethanol in a closed flask for 24 hours. Shaken frequently during the first 6 hours and allowed to stand for 18 hours. Then it was filtered rapidly without any loss of solvent. Evaporated 25 ml of the filtrate to dryness in a tarred flat-bottomed shallow dish and dried at 105°C, to constant weight. Calculated the percentage of alcohol soluble extractive with reference to air dried drug.

B. Water soluble extractive

Macerated 5 g of the air dried coarsely powdered drug with 100 ml of Chloroform water in a closed flask for 24 hours. Shaken frequently during the first 6 hours and allowed to stand for 18 hours. Then it was filtered without any loss of solvent. Evaporated 25 ml of the filtrate to dryness in a tarred flat-bottomed shallow dish and dried at 105 °C, to constant weight. Calculated the percentage of water soluble extractive with reference to air dried drug.

C. Ether soluble extractive

Macerated 5 g of the air dried, coarsely powdered drug with 100 ml of Diethyl ether in a closed flask for 24 hours. Shaken frequently during the first 6 hours and allowed to stand for 18 hours. Then it was filtered, without any loss of solvent. Evaporated 25 ml of the filtrate to dryness in a tarred flat-bottomed shallow dish and dried at 105 °C, to constant weight. Calculated the percentage ether soluble extractive with reference to air dried drug.

Loss on drying

It is the loss of weight expressed as percentage w/w. It determines the amount of volatile matter of any kind (including water) that can be driven off under the conditions specified.

Procedure

Accurately weighed about 1.5 g of fresh leaves of in a tarred porcelain dish and dried in an oven at 105°C to constant weight, cooled in desiccator and weighed. From the difference in weights percentage loss on drying was calculated.

Crude fibre

Crude fibre consist of the material other than ash which is insoluble in water and cannot be digested by boiling with dilute acid or alkali. Excess of crude fibre indicate the adulteration with woody tissues.

Procedure

Accurately weighed about 2 g of powdered drug in beaker and added 50 ml of 10% v/v Nitric acid. Heated to boil with constant stirring for 30 minutes. The mixture was then strained through fine cotton cloth and the residue was washed with hot water until free from acid. The residue was transferred to the beaker and added 50 ml 2.5% sodium hydroxide solution. Again, heated to boil with constant stirring for 30 minutes. Strained and washed with hot water as earlier. Transferred the residue in tarred crucible, dried, weighed and the percentage crude fibre content was calculated with reference to the air-dried drug.

Detection of inorganic constituents

To the prepared ash of the drug material, added 50% v/v Hydrochloric acid, kept for 1 hour and filtered. Then the following tests were performed on the filtrate.

a) **Test for nitrates:** Warmed the filtrate with few drops of conc. sulphuric acid and copper turnings.
b) **Test for chlorides:** To 3 ml of the filtrate added a few ml of 10% silver nitrate solution.
c) **Test for carbonates:** 2 ml of the filtrate was treated with few ml of mercuric chloride solution.
d) **Test for sulphates:** A few ml of filtrate was treated with 5% barium chloride solution.
e) **Test for Iron:** To 3 ml of the filtrate added a few ml of potassium ferrocyanide.

f) **Test for Calcium:** To 3 ml of filtrate added few drops of aqueous ammonia to make the solution basic and added saturated ammonium oxalate solution dropwise.
g) **Test for phosphates:** To the filtrate added nitric acid and a few drops of ammonium molybdate solutions, then heated for 10 minutes and cooled.

Fluorescence analysis of dried powder
Fluorescence analysis is an important tool for the screening of those compounds which have the property of exhibiting different colour under UV light. Some compounds are not fluorescent themselves but when they are treated with solvents or chemical reagents, get converted into fluorescent derivatives.

Procedure
The powdered leaves of *Procris repens* and *Sargassum duplicatum* was treated with freshly prepared reagents and analyzed under ordinary light, short and long ultraviolet wavelength to assess the fluorescent behavior and the results were tabulated.

Phytochemical screening
The whole plant or organism serves as an active laboratory for the production of natural products from primary metabolites. Primary metabolites are the products of vital metabolic pathways such as respiratory chain, TCA cycle etc. Secondary metabolites are varieties of simple to sophisticated bizarre molecules. They are fascinating chemical molecules, very useful and of great importance in nature, as well as highly diversified in structures, properties, uses, chemistry etc.

Extraction
The process of separating active principles from powdered crude drugs by using suitable solvents is called extraction. The choice of solvent depends upon the characteristics of the secondary metabolites like polarity, pH and thermal stability. Successive solvent extraction is suitable to extract the constituents of different polarity. It involves the extraction of the same plant material with solvents of different polarity ranging from non-polar to polar.

Soxhlet extraction
100 g of leaves of *Procris repens* and whole plant of *Sargassum duplicatum* were shade dried for seven days and size reduced by using a home mixer and extraction was carried out by continues hot extraction method using soxhlet apparatus with 1 liter of methanol in the round bottom flask and extraction was continued for 10 hr. The extract obtained was collected and concentrated by evaporator. The concentrated extract was weighed and stored.

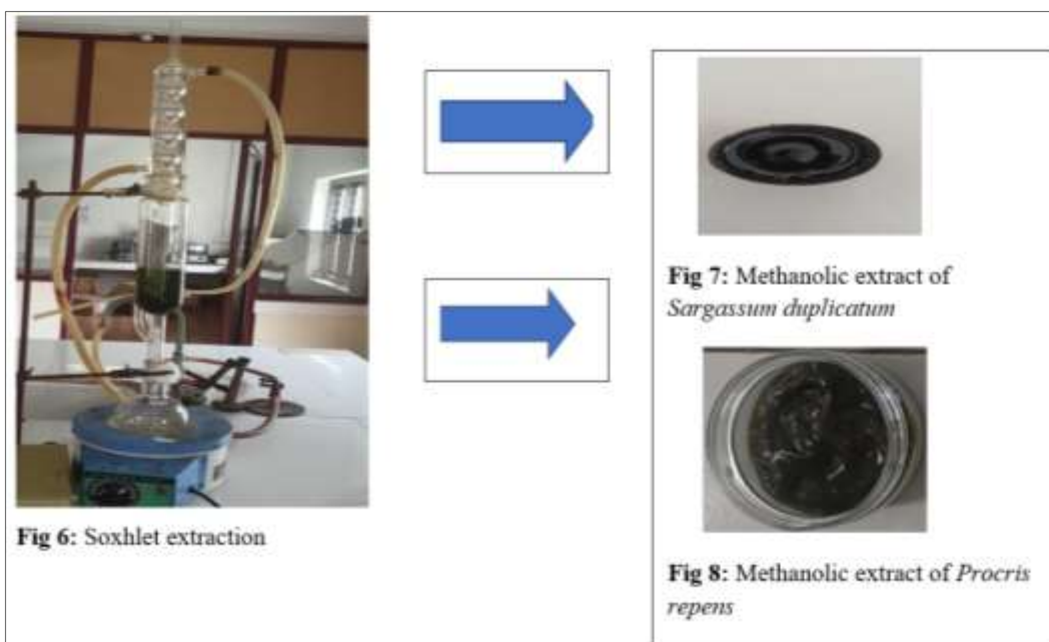

Fig 6: Soxhlet extraction

Fig 7: Methanolic extract of *Sargassum duplicatum*

Fig 8: Methanolic extract of *Procris repens*

A. Preliminary phytochemical screening
Preliminary phytochemical screening was done to identify different constituents present in extracts i.e. carbohydrates, proteins, lipids, flavonoid, tannins, glycosides, alkaloids, essential oils etc. All the extracts of *Procris repens* leaves and *Sargassum duplicatum* whole plant were subjected to preliminary phytochemical screening.

1. **Detection of Alkaloids**

a) **Mayer's test:** 2 ml of the extract was treated with 2 ml of Mayer's reagent gives cream precipitate.
b) **Dragendroff's Test**
2 ml of the extracts was treated with 2 ml of Dragendorff's reagent. Orange brown precipitate is formed.
c) **Hager's Test:** 2 ml of the filtrate was treated with Hager's reagent gives yellow precipitate
d) **Wagner's Test:** 2 ml of the filtrate was treated with few drops of Wagner's reagent gives brown precipitate.
e) **Tannic acid Test:** 2 ml of extract was treated with 2 ml of tannic acid solution gives yellow colour crystalline precipitate.

2. Detection of Carbohydrates

a. **Molisch's Test:** 1 ml of the test solution was mixed with 2 ml of Molisch reagent, shaken the mixture and added 1 ml of concentrated sulphuric acid along the sides of the test tube.Violet ring is formed at the junction of two liquids.
b. **Fehling's Test:** Boiled 1 ml of test solution with 1 ml Fehling's solution A and 1 ml Fehling's solution B on a water bath. First yellow, then brick red precipitate is observed.

c. **Benedict's test:** Mixed 2 ml of the Benedict's reagent with 2 ml of the test solution. Boiled in a water bath. Solution appears green, yellow or red depending on amount of reducing sugar present in test solution.
d. **Barfoed's Test:** Test solution treated with Barfoed's reagent and after boiling on water bath, it showed brick red color precipitate.
e. **Iodine Test:** Mixed 0.5 ml of Iodine solution with 1 ml of test solution showed deep blue colour.

3. Detection of proteins and amino acids

a. **Biuret test:** About 2 ml of extract was mixed with 2 ml of Biuret reagent shows purple colour.
b. **Millon's test:** 2 ml of the extract was mixed with 2 ml of Millon's reagent and boiled shows red coloured precipitate.
c. **Ninhydrin test:** Boiled 2 ml of the extract with 1ml of 5% ninhydrin solution shows purple coloured precipitate.
d. **Xanthoproteic test:** 2 ml of the extract was treated with 1ml of concentrated shows orange colour.

4. Detection of Steroids and triterpenoids

a. **Libermann test:** Mixed 2 ml of test solution with 2 ml of acetic anhydride and boiled. Then added 0.5 ml of concentrated sulphuric acid gives a deep green colour.
b. **Libermann- Burchard Reaction:** Mix 2ml extracts with chloroform, add 1-2 ml acetic anhydride and 2 drops conc. H2SO4 through the side of test tube. First red, then blue and finally green color appears.
c. **Salkowski Reaction:** To 2 ml. of extract add 2 ml. of chloroform and 2 ml. conc. H2SO4. Shake well. Chloroform layers appears red and acid layer shows yellow fluorescence.

5. Detection of Saponins

Foam test: 1ml solution of extract was diluted with distilled water to 20 ml and shaken in a graduated cylinder for 15 minutes gives development of stable foams.

6. Detection of glycosides

Preparation of test solution: The test solution was prepared by dissolving extract in the alcohol or hydro-alcoholic solution.

a. **Borntrager's test:** To a little quantity of sample solution added Sulphuric acid and carbon tetrachloride. Separated the organic layer and shaken with dilute ammonia. Appearance of pink to red colour indicate presence of anthraquinone moiety.
b. **Modified Borntrager's test (modified anthraquinone test for C-glycosides):** To little quantity of sample solution added ferric chloride solution, hydrochloric acid and Carbon tetrachloride. Separated the organic layer and shaken with dilute ammonia. Appearance of pink to red colour indicate the presence of Anthraquinone moiety.
c. **Legal's test:** To the extract add 1 ml of pyridine and 1 ml of sodium nitroprusside. A pink to red colour indicates the presence of glycosides.
d. **Baljet's test:** To a few ml of the extract, add 1 ml of sodium picrate solution. Yellow to orange colour reveals the presence of glycosides.

7. Detection of Tannins and phenolic compounds

a. **Ferric chloride test:** Mixed 2 ml of the test solution with few ml of 5% ferric chloride solution shows deep blue-black colour.
b. **Lead acetate test:** Mixed 2 ml test solution with 1 ml of lead acetate solution shows white precipitate.
c. **Potassium dichromate test:** Mixed 2 ml of the test solution with potassium dichromate solution changes from orange to green colour.
d. **Dilute Nitric acid test:** Mixed 2 ml of the test solution with dilute nitric acid shows colourless.
e. **Dilute iodine test:** Mixed 2 ml of the test solution with dilute iodine solution shows blue colour.
f. **Bromine water test:** Mixed 2 ml of the test solution with bromine water shows decolouration of bromine water.

8. Detection of Flavonoids

a. **Shinoda test:** To dry powder or extracts, add 5 ml. 95% ethanol, few drops of conc. HCl and 0.5 g magnesium turnings. Pink color observed.
b. **Sulphuric acid test:** Added few drops of concentrated sulphuric acid to few ml of sample solution shows yellow colouration that disappear on standing.
c. **Lead acetate test:** Mixed 2 ml of the test solution with lead acetate solution shows yellow colour precipitate.
d. **Alkali test:** Treated the test solution with increasing amount of sodium hydroxide shows yellow colouration, which decolourises after addition of acid indicates the presence of flavonoids.

9. Detection of mucilage

a. **Ruthenium red test:** Treated the powder with ruthenium red
b. **Swelling test:** Dissolved the powder in water.

10. Detection of fixed oils and fats

a. **Filter paper test:** Pressed the powder between filter paper.

Determination of total chlorophyll content

Chlorophyll is a green photosynthetic pigment found in plants. It absorbs sunlight and uses its energy to synthesize carbohydrates from carbon dioxide and water. There are two types of chlorophyll in plants, chlorophyll a and b which both of them works as photoreceptor in photosynthesis.

Materials required: Acetone, Fresh leaves

Procedure

One gram of finely cut fresh leaves of *Procris repens* were taken and ground with 20-40 ml of 80% acetone. It was then centrifuged at 5000-10000 rpm for 5 min. The supernatant was transferred to in a dry volumetric flask. The procedure was repeated till the residue becomes colorless. The absorbance of the solution was read at 645 and 663 nm against the solvent (acetone) blank. The concentrations of chlorophyll a, chlorophyll b and total chlorophyll were calculated using the following equation: (in µg/ ml)

Total chlorophyll – 20.2 (absorbance at 645) + 8.02 (absorbance at 663)

Chlorophyll a = 12.7 (absorbance at 663) – 2.69 (absorbance at 645)

Chlorophyll b = 22.9 (absorbance at 645) – 4.68 (absorbance at 663)

Chlorophyll 'a' mg/g tissue = 12.7 (A_{663}) - 2.69 (A_{645}) x V/(1000 x W)

Chlorophyll 'b' mg/g tissue = 22.9 (A_{645}) – 4.68 (A_{663}) x V/(1000 x W)

Total Chlorophyll (mg/g) = 20.2 (A_{645}) + 8.02 (A_{663}) x V/(1000 x W)

A = Absorption at specific wavelength
V = Final volume of extract with acetone
W = Fresh weight of tissue taken for extraction

Separation of Leaf Pigments by TLC
Principle
Plant pigments are organic substances responsible for colour of various plant parts like flowers, fruits and leaves. They are important to plants for photosynthesis and in development. Many of the plant pigments like carotenoids, betalains are useful to humans for therapeutic purposes. Commercially they are used as dyes, food and cosmetic colorants. Thin layer chromatography is also based on the principle of separation. The separation depends on the relative affinity of compounds towards stationary and the mobile phase. Once separation occurs, the individual components are visualized as spots at a different level of travel on the plate. Their nature or character is identified using suitable detection techniques.

TLC system components
TLC plates: These are stable and chemically inert plates, where a thin layer of stationary phase is applied on its whole surface layer. The stationary phase on the plates is of uniform thickness and is in fine particle size.

TLC chamber: This is used for the development of the TLC plate. The chamber maintains a stable environment inside for proper development of spots. It also prevents the evaporation of solvents and keeps the process dust-free.

Mobile phase: This comprises of a solvent or solvent mixture. The mobile phase used should be particulate-free and of the highest purity for proper development of TLC spots. The solvents recommended are chemically inert with the sample, a stationary phase.

Materials required: Fresh leaves, Acetone, Petroleum ether, Iso-propanol, Water

Procedure: Fresh green leaves of *Procris repens* were taken and grinded with acetone. Decanted the liquid and evaporated to ¼th of its original volume. Applied this leaf extract as spot on TLC plate. Developed the TLC plate in a chamber saturated with mobile phase Petroleum ether: isopropanol: water 100: 11: 5 drops. After complete development the plate was dried at room temperature and colours were observed and the pigments were identified.

Result and Discussion
Pharmacognostical studies
Macroscopy and microscopy of fresh leaves
Macroscopic character of *Procris repens*

Table 1: Macroscopic character of *Procris repens*

Sl. No.	Features	Observation
1.	Colour	Grey-green to dark green
2.	Odour	Aromatic
3.	Taste	Bitter and pungent
4.	Shape	Elliptic to oblong
5.	Size	2-8 cm x 1-4 cm
6.	Texture	Smooth, glabrous, slightly waxy
7.	Apex	Acute, Obtuse
8.	Margin	Crenate
9.	Venation	Pinnate
10.	Base	Rounded, attenuate
11.	Petiole	Absent

Macroscopic character of *Sargassum duplicatum*

Table 2: Macroscopic character of *Sargassum duplicatum*

Organoleptic Characters	Leaves	Axis	Holdfast
Colour	Yellow Brown	Dark Brown	Dark Brown
Odour	Briny/ Marine	Briny/ Marine	Briny/ Marine
Taste	Salty	Salty	Salty
Shape	Cup Shaped	Cylindrical	Disc Shaped
Size	5-10 Cm	15-30 Cm	1-10 Cm
Texture	Smooth Wavy	Rough, Flexible	Rough, Fibrous

Microscopical evaluation of leaf of *Procris repens*

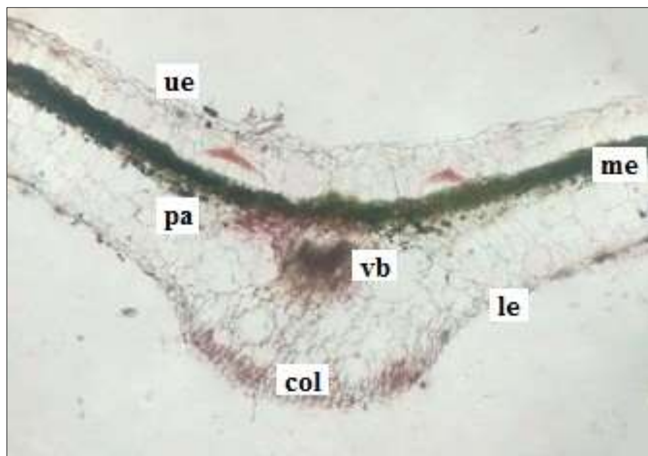

Fig 9: Ground plan of *Procris repens* (Leaves):

- col.: collenchyma cells.
- le.: lower epidermis.
- me.: mesophyll cells.
- pa.: parenchyma cells.
- ue.: upper epidermis.
- vb.: vascular bundles.

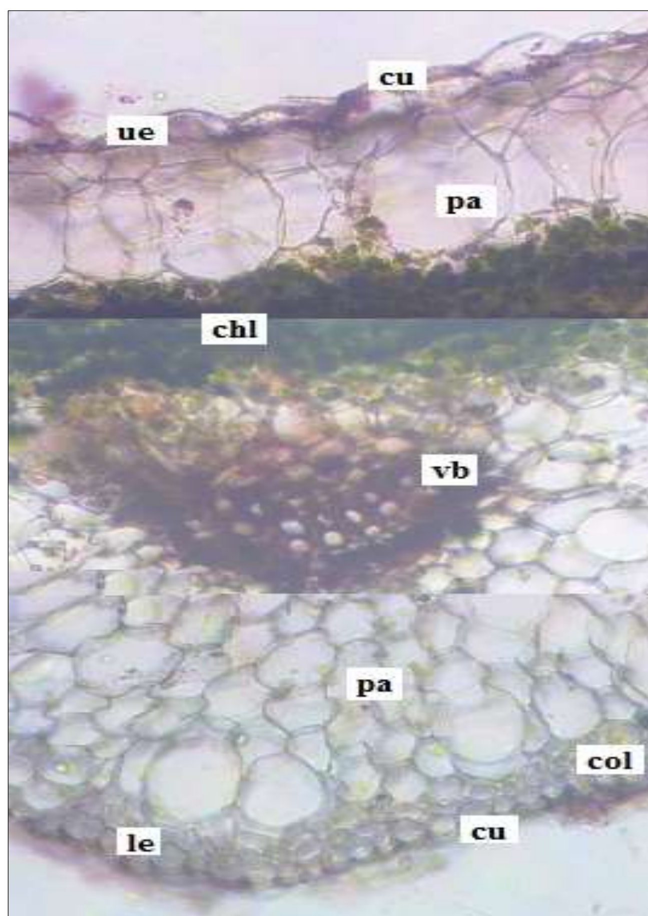

Fig 10: Portion enlarged of mid rib in *Procris repens* (leaves):

- chl.: chlorenchyma;
- col.: collenchyma cells;
- cu.: cuticle;
- le.: lower epidermis;
- pa.: parenchyma cells;
- ue.: upper epidermis;
- vb.: vascular bundles.

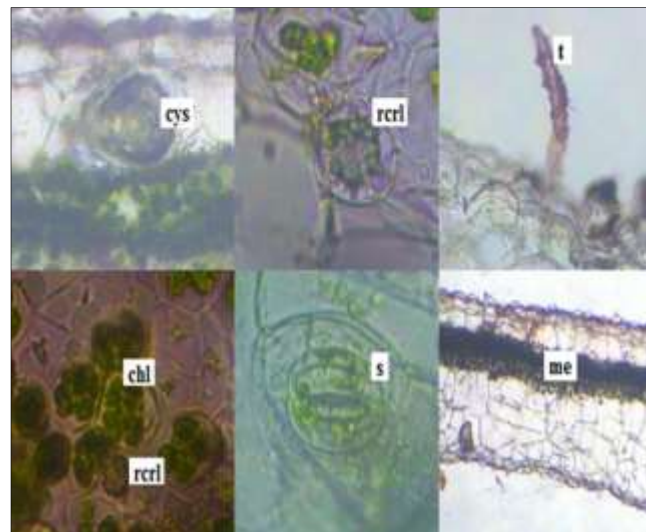

Fig 11: Transverse section of *Procris repens* (leaves):

- chl.: chlorenchyma cells.
- cys.: cystolith.
- me.: mesophyll cells.
- rcrl.: rosette crystals of calcium oxalate.
- s.: stomata.
- t.: trichome.

(b) Microscopy of stem

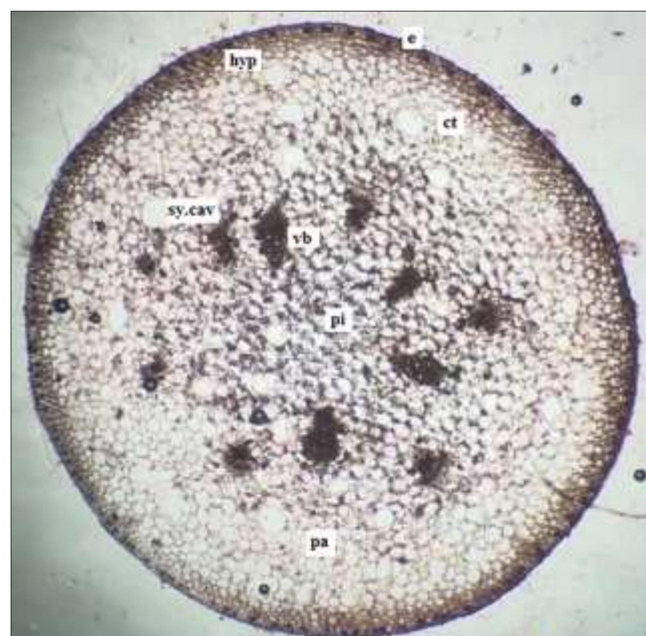

Fig 12: Ground plan of *Procris repens* (stem):

- e.: epidermis;
- hyp.: hypodermis;
- ct.: cortex;
- pa.: parenchyma cells;
- sy.cav.: schizogenous cavity;
- vb.: vascular bundles;
- pi.: pith.

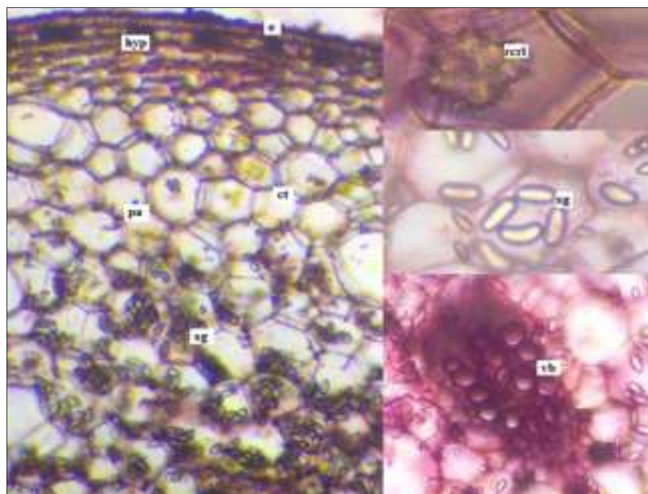

Fig 13: Transverse section of *Procris repens* (stem) a portion enlarged:

- e.: epidermis;
- hyp.: hypodermis;
- ct.: cortex;
- pa.: parenchyma cells;
- rcrl.: rosette crystals of calcium oxalate;
- vb.: vascular bundles;
- sg.: starch grains.

(c) Microscopy of root

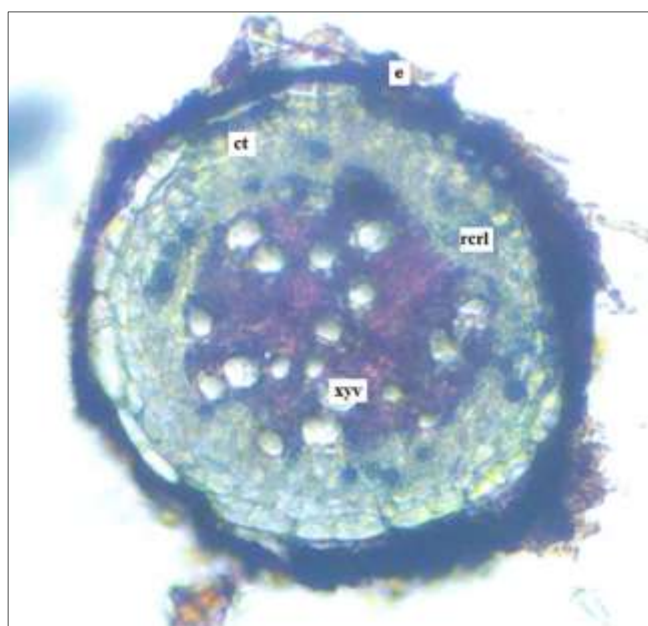

Fig 14: Ground plan of *Procris repens* (Root):

- ck.: cork;
- ct.: cortex;
- rcrl.: rosette crystals of calcium oxalate;
- xyv.: xylem vessel.

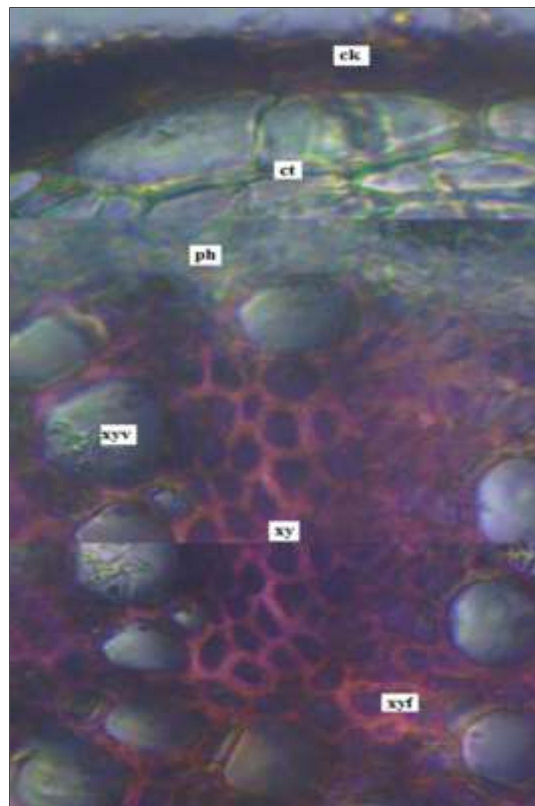

Fig 15: Transverse section of *Procris repens* (Root) a portion enlarged:

- ck.: cork;
- ct.: cortex;
- ph.: phloem;
- xy.: xylem;
- xyv.: xylem vessel;
- xyf.: xylem fiber.
- Powdermicroscopy

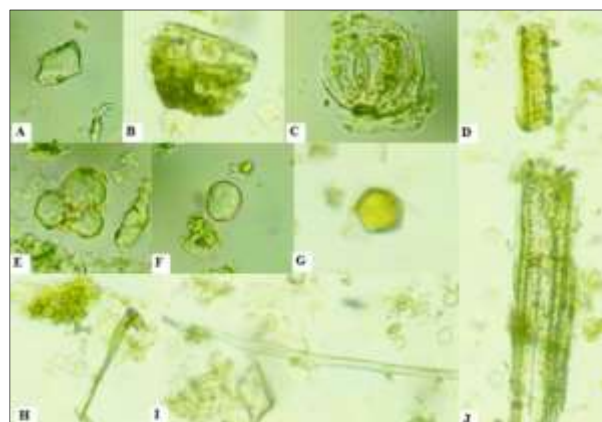

Fig 16: Powder microscopy; A.: crystal of calcium oxalate; B.: fragment of mesophyll cells; C.: stomata; D.: spiral vessel; E.: parenchyma cells; F.: starch grains; G.: coloured content; H.: fragment of epidermal cells with trichome; I.: non-lignified fiber; J.: vascular element with bordered pitted vessels and annular vessels.

Microscopical evaluation of blades of *Sargassum duplicatum*

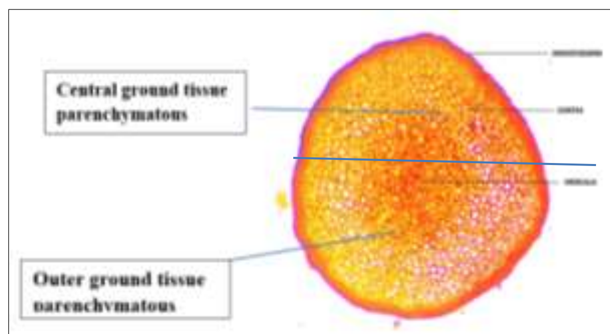

Fig 17: TS of Blades of *Sargassum duplicatum*

Stain: Safranin and Magnification: 45 x

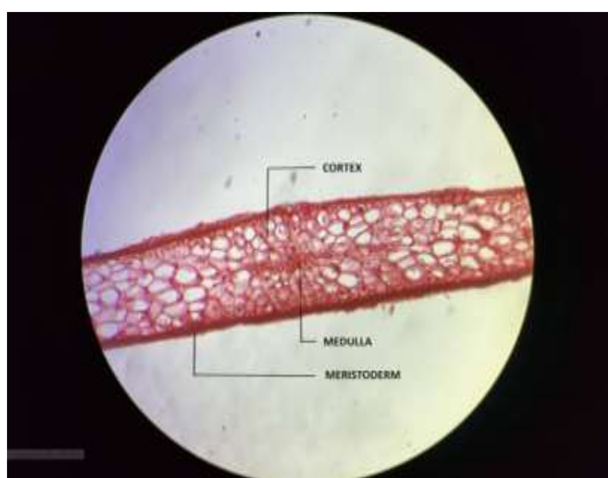

Fig 18: TS of blades a sector enlarged of *Sargassum duplicatum*

Stain: Safranin and Magnification: 10 x

Determination of physicochemical constants

Table 3: Determination of physicochemical constants

Parameter	*Procris repens*	*Sargassum duplicatum*
ASH value Total ash	3.84 ± 0.0013% w/w	7.15 ± 0.0012% w/w
Acid insoluble ash	1.24 ± 0.0020% w/w	3.65 ± 0.0013% w/w
Water soluble ash	2.43 ± 0.0010% w/w	2.14 ± 0.0021% w/w
Sulphated ash extractive value	7.82 ± 0.0010% w/w	10.92 ± 0.0014% w/w
Ether soluble extractive	0.69 ± 0.0015% w/w	5.45 ± 0.0020% w/w
Alcohol soluble extractive	1.8 ± 0.020% w/w	10.45 ± 0.001% w/w
Water soluble extractive	0.8 ± 0.0425% w/w	2.32 ±0.0012% w/w
Loss on drying	1.09 ± 0.0057% w/w	7.45 ± 0.0041% w/w
Crude fiber content	12 ± 0.011% w/w	10 ± 0.002% w/w
Foreign matter	0.8% w/w	0.4% w/w

Detection of inorganic constituents

Table 4: Detection of inorganic constituents

Sl. No.	Experiment	Procris repens extract	Sargassum duplicatum extract
1.	Test for nitrates	Absent	Present
2.	Test for calcium	Present	Present
3.	Test for phosphates	Present	Present
4.	Test for iron	Present	Present
5.	Test for Sulphates	Present	Present
6.	Test for Chlorides	Present	Present
7.	Test for Carbonates	Absent	Present

Fluorescence analysis of dried powder

Table 5: Fluorescence analysis of dried powder of *Procris repens*

SL. No	Treatment	Day light	UV 254NM	UV 365nm
1.	Dry powder	Gray-green	Yellowish green	Dark green
2.	Powder + 1N HCl	Gray- green	Yellowish green	Brown
3.	Powder +1N H2SO4	Gray- green	Yellowish green	Brown
4.	Powder + 1N HNO3	Gray- green	Yellowish green	Brown
5.	Powder + 1N NaOH	Gray- green	Green	Greenish brown
6.	Powder + Alc. KOH	Gray- green	Green	Greenish brown
7.	Powder + Picric acid	Greenish Yellow	Yellow	Brownish yellow
8.	Powder +FeCl3 (5%)	Brown	Brown	Greenish brown
9.	Powder + 1% iodine	Yellowish green	Yellowish green	Brown
10.	Powder + Dil. KMnO4	Violet	Greenish violet	Violet
11.	Powder + Acetic acid	Greenish Brown	Brown	Brown
12.	Powder + acetone	Green	Green	Yellowish green
13.	Powder + Ethanol	Yellowish green	Green fluorescence	Brown
14.	Powder + FeCl3	Reddish brown	Brown	Brown
15.	Powder + 50%H2SO4	Green	Pale green	Dark brown
16.	Powder + Con.H2SO4	Reddish brown	Brown	Black
17.	Powder +Con. HCl	Yellowish green	Brown	Black
18.	Powder + Con HNO3	Reddish brown	Brown	Black
19.	Power + dil. NH3	Gray- green	Green	Brown
20.	Powder + Na2CO3	Gray- green	Dark green	Brown

Table 6: Fluorescence analysis of dried powder of *Sargassum duplicatum*

SL. No	Treatment	Day light	UV 254NM	UV 365nm
1.	Dry powder	Light brown	Dark brown	Greenish yellow
2.	Powder + 1N HCl	Light brown	Dark green	Brown
3.	Powder +1N H2SO4	Light brown	Dark green	Brown
4.	Powder + 1N HNO3	Light brown	Dark green	Brown
5.	Powder + 1N NaOH	Light brown	Green	Greenish brown

6.	Powder + Alc. KOH	Light brown	Green	Greenish brown
7.	Powder + Picric acid	Dark yellow	Yellow	Brownish yellow
8.	Powder +$FeCl_3$ (5%)	Brown	Brown	Greenish brown
9.	Powder + 1% iodine	Dark brown	Dark green	Brown
10.	Powder + Dil. $KMnO_4$	Sandal	Sandal	Sandal
11.	Powder + Acetic acid	Greenish brown	Brown	Brown
12.	Powder + acetone	Green	Green	Dark green
13.	Powder + Ethanol	Light green	Green fluorescence	Brown
14.	Powder + $FeCl_3$	Reddish brown	Brown	Brown
15.	Powder + 50%H_2SO_4	Sandal	Pale green	Dark brown
16.	Powder + Con.H_2SO_4	Reddish brown	Brown	Black
17.	Powder +Con. HCl	Dark green	Brown	Black
18.	Powder + Con HNO_3	Reddish brown	Brown	Black
19.	Power + dil. NH_3	Light brown	Brown	Brown
20.	Powder + Na_2CO_3	Dark brown	Dark brown	Brown

Phytochemical screening
Preliminary phytochemical screening

Table 7: Preliminary phytochemical screening

Sl. No.	Qualitative tests	Methanolic extract of *Procris repens*	Methanolic extract of Sargassum duplicatum
1.	**Test for Alkaloids**		
	Mayer's test	+	+
	Dragendroff's test	+	+
	Hagers test	+	+
	Wagner's test	+	+
	Tannic acid test	+	+
2.	**Test for carbohydrates**		
	Molisch's test	-	+
	Fehling's test	-	+
	Benedict's test	-	+
	Barfoed's test	-	+
	Iodine test	-	-
	Seliwanoff's test	-	+
3.	**Test for protein and amino acids**		
	Biuret test	-	-
	Millon's test	-	-
	Ninhydrin test	-	-
	Xanthoprotein test	-	-
4.	**Test for steroids and triterpenoids**		
	Liebermann test	+	+
	Libermann Burchard	+	+
	Salkowski test	+	+
5.	**Test for saponins**		
	Foam test	+	+
6.	**Test for glycosides**		
	Borntrager's test	+	+
	Modified Borntrager's test	+	+
	Legal test	+	+
	Baljet test	+	+
7.	**Tests for Tannins - phenolic compounds**		
	Ferric chloride test	+	+
	Lead acetate test	+	+
	Potassium dichromate test	+	+
	Dil.HNO_3 test	+	+
	Dil. iodine test	+	+
	Bromine water test	+	+
8.	**Tests for flavonoids**		
	Shinoda test	+	+
	Sulphuric acid test	+	+
	Lead acetate test	+	+
	Alkali test	+	+
9.	**Tests for mucilage**		
	Ruthenium red test	-	-
	Swelling test	-	-
10.	**Tests for fixed oils and fats**		
	Filter paper test	-	-

Determination of total chlorophyll content

Table 8: Absorbance of chlorophyll a and b in *Procris repens*

Sample code	OD_{645nm}	OD_{663nm}	Volume of acetone (ml)	Weight of sample (g)
PRL	0.651	1.352	5	0.1

Table 9: Amount of chlorophyll a and b in the leaves of *Procris repens*

Sample code	Chlorophyll a		Chlorophyll b		Total chlorophyll	
	(µg/ml)	(mg/g)	(µg/ml)	(mg/g)	(µg/ml)	(mg/g)
A	15.42	0.771	8.58	0.429	23.99	1.20

Separation of leaf pigments of *Procris repens* by thin layer chromatography

Fig 19: TLC before spraying

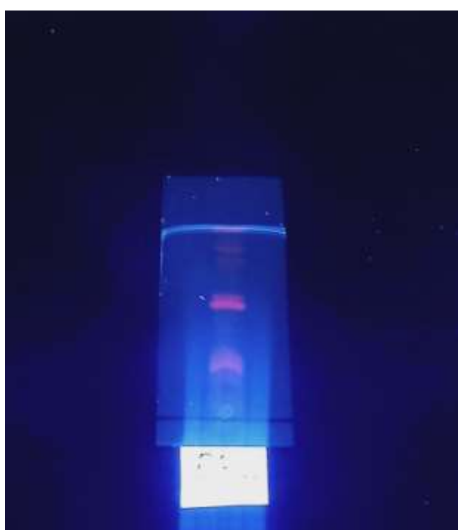

Fig 20: TLC after spraying

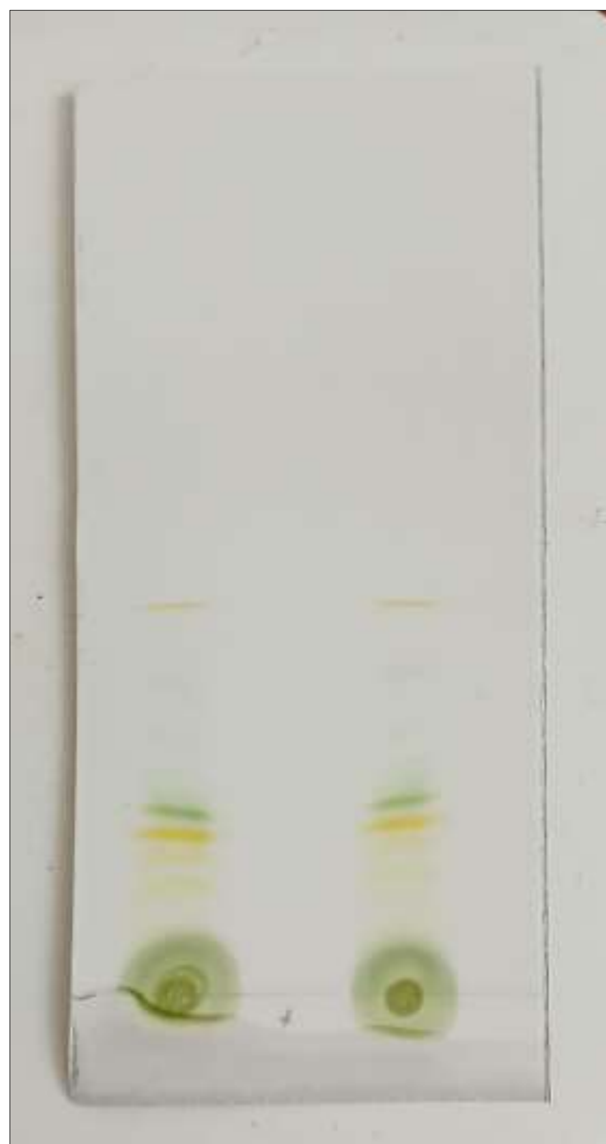

Fig 21: Separated leaf pigments

Sample code	Biomolecule	Mobile Phase – (Solvent System Used)	Proportion of Solvent system
PRL	Leaf Pigments	Petroleum Ether: Isopropanol: Water	5:0.55:0.25

Biomolecule	Sample code	Bands	Colour of Band (visible light)	Solvent front (cm)	Solute front (cm)	Rf value
Leaf Pigments	PRL	1	Light Yellow	6	1.6	0.26
		2	Light green		2.3	0.38
		3	Green		4.7	0.78
		4	Bluish Green		5.1	0.85
		5	Dark Yellow		5.7	0.95

Table 8: Leaf pigments – colour and R_f value

Sl. No.	Pigment	Colour	R_f value
1.	Xanthophyll	Light yellow	0.26
2.	Chlorophyll b	Light green	0.38
3.	Chlorophyll a	Green	0.78
4.	Phaeophytin	Yellow- grey	0.83
5.	Carotene	Dark yellow	0.95

Conclusion

In the present investigation, selected *Procris repens* plant belonging to terrestrial and *Sargassum duplicatum* seaweed from marine source. This research paper gives information about the Pharmacognostical and preliminary phytochemical screening of selected plants. The *plant* is used by Malays for poulticing boils, swollen areas, and the abdomen when it is painful.A decoction from the plant is used for rheumatism. *Procris repens* (Lour.) B. J. Conn and Hadiah(*Urticaceae*)is traditionally used in folk medicine for the treatment of skin injuries and respiratory conditions. Whole plant of *Procris repens* was used to treat icterus, acute and chronic hepatitis, and allergic dermatitis in Chinese medicine. *Sargassum duplicatum (S. duplicatum)* is one of the main seaweeds in the marine ecosystem that has been used as a source of medicine among brown seaweeds. These brown algae belong to the family *Sargassaceae.Sargassum duplicatum is used as a natural fertilizer and soil conditioner.It has the potential to be used in bioremediation efforts, helping to absorb and remove pollutants from aquatic environments. It has reported to posess anti cancer, anti arthritic* immunostimulatory, anti-diabetic, anti-obesity, antiviral activities. Pharmacognostical investigation includes the macroscopy, microscopy and powder microscopy. Here in macroscopy determine the color, odour, taste, shape, size and texture. Microscopy identify the parts such as collenchyma cells, lower epidermis, mesophyll cells, parenchyma cells, upper epidermis, vascular bundles, chlorenchyma, cuticle, hypodermis, cortex, schizogenous cavity, rosette crystals of calcium oxalate, starch grains And anisocytic stomata. Phytochemical screening of *Procris repens* found to contain alkaloids, steroids, triterpenoids, saponins, glycosides, tannins, phenolic compounds and flavonoids while in addition to it *Sargassum duplicatum* contain carbohydrate also.

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